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Quantification of cobimetinib, cabozantinib, dabrafenib, niraparib, olaparib, vemurafenib, regorafenib and its metabolite regorafenib M2 in human plasma by UPLC–MS/MS

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Abstract

A sensitive and selective ultra-high performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) method for the simultaneous determination of seven oral oncolytics (two PARP inhibitors, i.e. olaparib and niraparib, and five tyrosine kinase inhibitors, i.e. cobimetinib, cabozantinib, dabrafenib, vemurafenib and regorafenib, plus its active metabolite regorafenib M2) in EDTA plasma was developed and validated. Stable isotope-labelled internal standards were used for each analyte. A simple protein precipitation method was performed with acetonitrile. The LC–MS/MS system consisted of an Acquity H-Class UPLC system, coupled to a Xevo TQ-S micro tandem mass spectrometer. The compounds were separated on a Waters CORTECS UPLC C18 column (2.1 × 50 mm, 1.6 μm particle size) and eluted with a gradient elution system. The ions were detected in the multiple reaction monitoring mode. The method was validated for cobimetinib, cabozantinib, dabrafenib, niraparib, olaparib, vemurafenib, regorafenib and regorafenib M2 over the ranges 6–1000, 100–5000, 10–4000, 200–2000, 200–20,000, 5000–100,000, 500–10,000 and 500–10,000 μg/L, respectively. Within-day accuracy values for all analytes ranged from 86.8 to 115.0% with a precision of <10.4%. Between-day accuracy values ranged between 89.7 and 111.9% with a between-day precision of <7.4%. The developed method was successfully used for guiding therapy with therapeutic drug monitoring in cancer patients and clinical research programs in our laboratory.

KEYWORDS

cabozantinib, cobimetinib, dabrafenib, niraparib, olaparib, quantification method, regorafenib, therapeutic drug monitoring, UPLC–MS/MS, vemurafenib

1 | INTRODUCTION

Over the last two decades, the development of targeted oral anticancer drugs has increased strongly and this is expected to continue. After the approval of the first oral tyrosine kinase inhibitor imatinib in 2001, various oral kinase inhibitors have been approved, specifically targeting one or multiple protein kinases (Dagher et al., 2002; Roskoski, 2019). Protein kinases play a key role in activating proteins that are involved in signal transduction pathways that regulate cell survival, proliferation and differentiation. In patients with

malignancies, these pathways are often upregulated as they drive tumour growth, proliferation and angiogenesis (Ardito, Giuliani, Perrone, Troiano, & Lo Muzio, 2017). Hence, inhibitors of protein kinases in these pathways comprise an important therapeutic intervention (Zhang, Yang, & Gray, 2009). Protein kinase inhibitors that have been approved recently and are used in our clinic include vemurafenib, dabrafenib, cobimetinib, regorafenib and cabozantinib. Recently, a new group of targeted oral anticancer drugs was introduced. Olaparib and niraparib are inhibitors of the poly ADP ribose polymerase 1 (PARP-1). PARP is essential for the repair of single-strand DNA breaks via the base excision pathway. Inhibiting PARP results in double-strand DNA breaks which result in cell death (Ashworth, 2008). Although PARP inhibitors were initially intended for use in cancers driven by BRCA1 or 2 mutations, these drugs are now also being investigated for use in homologous repair-deficient tumors lacking BRCA1 and 2 mutations and in combination with chemotherapy or radiation to enhance the DNA-damaging effects (Cesaire et al., 2018; Lu, Liu, Pang, Pacak, & Yang, 2018).

PARP inhibitors and tyrosine kinase inhibitors are registered in a fixed dose, which means each patient receives the same dose regardless of body size differences. For some of these drugs, a clear relationschip between drug exposure and efficacy has already been described. For instance, for patients treated with cabozantinib, greater antitumour acitivity was observed for patients with a steady-state concentration >750 μg/L (Lacy et al., 2018). For vemurafenib, a lower risk of disease progression was seen for patients with a median plasma concentration of 42,000 μg/L during the first year of treatment (Goldwirt et al., 2016). Patients treated with these drugs will probably benefit from routine therapeutic drug monitoring to achieve these target levels. For the other drugs, the relationship between drug exposure and response needs to be further elucidated. In addition, measuring the exposure of these drugs can be of help for dose adjustment decisions in the presence of drug–drug interactions or co-morbidities, as these drugs have a narrow therapeutic window and high inter-patient variability.

Therefore, there is a need for pharmacokinetic evaluation both in clinical studies and for individual patients. Our laboratory has previously implemented a bioanalytical method for the measurement of imatinib, sunitinib, desethyl sunitinib and pazopanib in a single run for routine patient care and clinical studies (van Erp et al., 2013). Since novel oral oncolytics have become available, an additional method had to be developed to analyse these drugs, preferably in a single run.

Numerous LC–MS/MS methods have been described for quantification of the individual compounds or a combination of cobimetinib (Cardoso et al., 2018; Deng et al., 2014; Huynh et al., 2017; Rousset et al., 2017), cabozantinib (Abdelhameed, Attwa, & Kadi, 2017), dabrafenib (Cardoso et al., 2018; Huynh et al., 2017; Merienne et al., 2018; Rousset et al., 2017), niraparib (van Andel et al., 2017), olaparib (Nijenhuis, Lucas, Rosing, Schellens, & Beijnen, 2013; Pressiat et al., 2018), vemurafenib (Cardoso et al., 2018; Huynh et al., 2017; Nijenhuis, Rosing, Schellens, & Beijnen, 2014; Rousset et al., 2017) and regorafenib (Cardoso et al., 2018; Huynh et al., 2017; Luethi et al., 2014; Merienne et al., 2018; van Erp et al., 2013) in human plasma. However, a quantification method combining all of the above mentioned oncolytics in a single run has not been published yet.

Our objective was to develop and validate a sensitive and selective bioanalytical method by ultra-high performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) for the simultaneous quantification of seven targeted oral oncolytics (cobimetinib, cabozantinib, dabrafenib, niraparib, olaparib, vemurafenib and regorafenib plus its metabolite regorafenib M2) in human EDTA plasma.

2 | MATERIAL AND METHODS

2.1 | Chemicals and reagents

Regorafenib (RGF), regorafenib M2 (RGF M2), olaparib (OPR), vemurafenib (VMF), cobimetinib (CBT), niraparib (NPR), cabozantinib (CBZ) and dabrafenib (DBF) were obtained from Bio-Connect BV (Huissen, The Netherlands). The isotopes $^{13}C^{2}H_{3}$-regorafenib, $^{2}H_{8}$-olaparib, $^{13}C_{6}$-vemurafenib, $^{13}C_{6}$-cobimetinib, $^{13}C_{6}$-niraparib, $^{2}H_{4}$-cabozantinib and $^{2}H_{9}$-dabarefenib used as internal standards, were acquired from Alsachim (Illkirch, France). Dimethyl-sulfoxide (DMSO, Seccosolv) and acetonitrile (ACN, Lichrosolv) were purchased from Merck (Darmstadt, Germany). Formic acid was obtained from Sigma-Aldrich (Zwijndrecht, The Netherlands). High-purity Milli-Q water was produced using a MilliQ Gradient water purification system (Millipore, Amsterdam, The Netherlands). Ethylenediaminetetraacetic acid (EDTA) plasma was prepared from EDTA whole blood obtained from Sanquin (Amsterdam, The Netherlands).

2.2 | Chromatographic conditions

The LC–MS/MS system consisted of an Acquity H-Class UPLC system, coupled to a Xevo TQ-S Micro Tandem Mass Spectrometer (Waters, Wilford, MA, USA). Chromatographic separation was performed by injecting 10 μL supernatant onto a Cortecs UPLC C18 column (2.1 × 50 mm, 1.6 μm particle size, Waters). Mobile phase A consisted of 0.1% formic acid in water (Milli-Q) and mobile phase B consisted of 0.1% formic acid in ACN. The following gradient was used (time: %A/%B): 0–0.1 min: 80/20 5.0 min: 50/50 6.0 min: 10/90 6.0–7.0 min: 80/20. The flow rate was 0.8 ml/min. The column temperature was kept at 50°C and the autosampler temperature at room temperature (25°C). The LC eluate was directed into a tandem quadruple, atmospheric pressure ionization mass spectrometer (TQ-S detector, Acquity, Waters, Milford, MA, USA) equipped with an electrospray ionization source.

2.3 | Mass spectrometric conditions

The mass spectrometer was run in the positive ion mode and configured in multiple reaction monitoring mode for detection of RGF, RGF

M2, OPR, VMF, CBT, NRP, CBZ, DBF and their isotope-labelled analogues. Figure 1 shows the chemical structures of all eight analytes and their selected mass transitions and proposed *m/z* fragments.

Capillary voltage, cone voltage, collision energy and dwell time were optimized using Masslynx™ Intellistart Software (version 4.1, Waters, Etten-Leur, The Netherlands). The following settings for the Xevo TQ-S micro mass spectrophotometer were used: source temperature 150°C, desolvation temperature 500°C, nitrogen gas flow 1000 L/h and capillary voltage 4 kV. MS settings are shown in Table 1.

2.4 | Preparation of stock solutions, calibration standards, quality control samples and internal standard solution

Stock solutions were prepared in DMSO at a nominal concentration of 1000 mg/L (RGF, RGF M2, CBT, NRP, CBZ and DBF) or 10,000 mg/L (OPR and VMF). A series of eight working solutions for each analyte, except CBZ, was prepared by diluting the stock solutions in DMSO. During development, the limit of quantification of cabozantinib was expanded from 300 to 100 µg/L to cover the full range of clinically relevant plasma concentrations. Consequently, for cabozantinib, nine working solutions were prepared by diluting the stock solutions in DMSO. The preparation of the working solutions is shown in Supplementary Table 1. These working solutions were diluted 10-fold in human EDTA plasma to yield the concentrations of the calibrations curve as listed in the Table 2.

Quality control (QC) samples were prepared in a similar way, using stock solutions independently prepared from the stock solutions used for the calibration samples. For cabozantinib an additional extra low quality control sample (QCXL) was included. The concentrations of the QC samples in human EDTA plasma are listed in Table 2.

The internal standard stock solutions were prepared in DMSO at a nominal concentration of 1000 mg/L for each isotope-labelled analyte. For the precipitation solutions to which the internal standards are added, the isotope-labelled stock solutions of CBT and NRP were diluted 10-fold. Subsequently, precipitation solutions were prepared

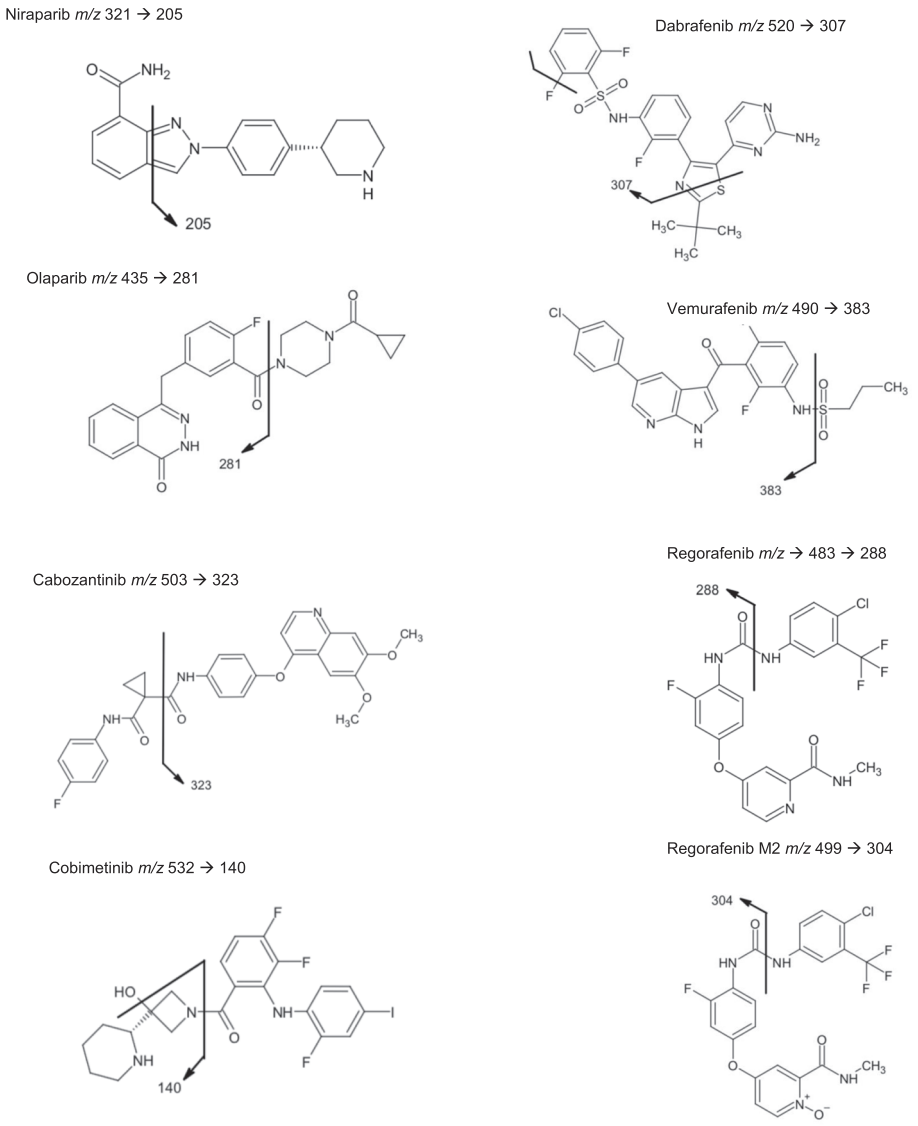

FIGURE 1 Chemical structures and proposed *m/z* fragments of all eight analytes

TABLE 1 Analyte and IS specific mass spectrometric parameters and optimized mass spectrometer settings

	Scheduled multiple reaction monitoring time (min)		Analyte (*m/z*)		Internal standard (*m/z*)				
	Start	End	Precursor (Q1)	Product Ion (Q3)	Precursor (Q1)	Product Ion (Q3)	Dwell (s)	Cone (V)	Collision (V)
NRP	0.00	0.75	321	205	327	211	0.099	44	40
OPR	0.75	1.75	435	281	443	281	0.099	36	30
CBZ	1.75	2.75	502	323	506	323	0.037	40	36
CBT	1.75	2.75	532	140	538	140	0.060	54	18
DBF	3.25	4.50	520	307	529	316	0.024	72	36
RGF M2[a]	3.25	4.50	499	304	487	292	0.024	36	36
VMF	4.50	5.50	490	383	496	389	0.017	90	26
RGF	4.50	5.50	483	288	487	292	0.017	56	22

Abbreviations: RGF, regorafenib; RGF M2, regorafenib M2; OPR, olaparib; VMF, vemurafenib; CBT, cobimetinib; NPR, niraparib; CBZ, cabozantinib; DBF, dabrafenib.
[a]For RGF M2, isotope-labelled RGF was used as an internal standard.

by adding 80 µl (CBZ, DBF, NRP), 160 µl (RGF, CBT) and 400 µl (OPR, VMF) of the internal standard stock solutions of the isotope-labelled compounds to 100 ml ACN.

2.5 | Sample preparations

Samples were mixed for 5 min and subsequently centrifuged for 5 min at 19,000***g***. Protein precipitation as sample preparation was performed by adding 200 µl of the precipitation reagent to 50 µl of EDTA plasma into a 1.5 ml polypropylene microcentrifuge tube. After vortex-mixing for 2 min, samples were centrifuged for 5 min at 19,000***g***. A volume of 20 µl of the supernatant was transferred to an autosampler vial, diluted 10-fold with water and vortex-mixed for 5 min. Subsequently, 10 µl was injected into the UPLC–MS/MS.

TABLE 2 Preparation of calibration standards and quality control samples

Analyte	Calibration (µg/L)	Quality control (µg/L) QCH; QCM; QCL; QCXL
RGF	10,000; 8300; 6640; 5000; 3500; 2000; 1000; 480	7500; 4500; 1500
RGF-M2	10,000; 8300; 6640; 5000; 3500; 2000; 1000; 480	7500; 4500; 1500
OPR	20,000; 16,600; 13,200; 10,000; 6800; 4000; 2000; 200	15,000; 9000; 600
VMF	100,000; 83,400; 66,800; 50,000; 35,000; 20,000; 10,000; 4800	75,000; 44,000; 15,000
CBT	1000; 840; 640; 500; 340; 200; 100; 6	740; 400; 20
DBF	4000; 3340; 2640; 2000; 1340; 800; 400; 10	3000; 1500; 30
CBZ	5000; 4160; 3320; 2500; 1840; 1000; 500; 500; 100	3760; 2000; 1500; 300
NRP	2000; 1660; 1320; 1000; 760; 400; 200; 300	1500; 800; 400

2.6 | Validation procedures

Method validation was performed in accordance with the "Guideline on bioanalytical method validation" of the European Medicines Agency (EMA) (EMA, 2012).

2.6.1 | Selectivity and carryover

Interference from endogenous compounds was investigated by analysing blank human EDTA plasma samples of six different individuals. Absence of interfering components was accepted when the response was <20% of the lower limit of quantification (LLOQ) for all analytes and <5% for the IS.

Carryover was assessed by injecting a blank human EDTA plasma sample without IS after injection of the higher limit of quantification (HLOQ) containing all eight analytes and IS. This step was repeated five times. To meet the requirements of the EMA guidelines, carryover in the blank sample should be <20% of the LLOQ of each drug and <5% of the IS.

2.6.2 | Accuracy and precision

Accuracy and within-day and between-day precision were determined by analysing spiked EDTA plasma samples at the LLOQ and HLOQ in addition to three different QC levels (H-M-L) in 5-fold on three different days. For cabozantinib QCXL was also included in this analysis.

The accuracy was calculated as the average percentage of the nominal concentration. For the within-day precision the highest coefficient of variation (CV) of the three runs was used. One-way analysis of variance (ANOVA) was used to assess the between-day precision for each of the five concentrations. The error mean square or mean square within groups (ErrMS), the day mean square or mean square among groups (DayMS), and the grand mean (GM) of all 15 measurements across the three run days were obtained from the ANOVA. The estimate of the between-day precision at every concentration was calculated as follows, in which n is the number of replicate measurements within each day:

$$\text{Between-day precision} = ([(\text{DayMS} - \text{ErrMS})/n]^{0.5}/\text{GM}) \times 100\%.$$

The within-day and between-day precision was expressed as relative standard deviation (RSD). For the lower limit of quantification, the percent deviation from the nominal concentration and the RSD should be <20%. For all other concentrations the percentage deviation from the nominal concentration and the RSD should be <15%.

2.6.3 | Extraction recovery

Total extraction recovery was determined for all analytes by comparing response ratios of extracted plasma samples with those obtained by direct injection of the same amount of drug in mobile phase at three concentrations (QCH, QCM and QCL) in duplicate. For cabozantinib total extraction recovery was additionally determined for QCXL in duplicate. According to our internal aim, the recovery ratios should be >70% and preferably be constant over the concentration range.

2.6.4 | Dilution integrity

Dilution integrity was investigated for samples with concentrations above the calibration range by analysing samples at a concentrations 1.5 times the HLOQ. Samples were diluted 2 and 4 times, respectively, with blank EDTA plasma. Each dilution was carried out 5-fold and compared with the nominal concentration. Accuracy and precision should be <15%.

2.6.5 | Matrix effect

The matrix effect was determined for all eight components and the labelled IS in six different blank EDTA plasma batches from individual donors. After precipitation with acetonitrile samples were spiked with the compounds at two concentrations (QCL and QCH) and the IS. The matrix factor (MF) was defined by calculating the ratio of the peak area in the presence and absence of matrix. The coefficient of variation (CV) of the IS normalized MF, calculated by dividing the MF of the components by the MF of the IS, should be within 15%.

2.6.6 | Stability

Stability of the stock solutions in DMSO was tested at −40°C. Spiked samples at three concentrations (QCL, QCM, QCH) were used for determining the stability in plasma (−40°C, 4°C and room temperature). Stability during sample handling was verified by subjecting a range of spiked samples to three freeze–thaw cycles (stored at −40°C). Additionally, autosampler stability over the range of LLOQ to HLOQ of processed samples (4°C) was tested. Stability of individual patient samples was determined in samples that were collected for routine patient care and were stored at −40°C after the initial analysis. Samples within the described limits of accuracy (±15%) were considered to be stable.

3 | RESULTS

3.1 | Method development

The chromatographic separation for the eight analytics is shown in Figure 2. Figure 2 shows the reconstructed ion chromatogram overlay for the medium calibration sample of the eight analytes. This clearly depicts the wide range of signal intensity, mainly caused by differences in concentrations measured, encountered in this integrated method. Run time for the final assay was 7 min.

3.2 | Method validation

3.2.1 | Calibration curve

RGF, RGF M2, OPR, VMF, CBT, NRP, CBZ and DBF were quantified in plasma by describing the peak area ratio to the internal standard vs. the nominal concentration. A quadratic curve with $1/x$ as weighting factor proved to result in the best fit. The range of the calibration curve was chosen to cover the expected clinically relevant plasma concentrations. The calibration range covers the range of 500–10,000 μg/L for regorafenib and regorafenib M2, 200–20,000 μg/L for olaparib, 5,000–100,000 μg/L for vemurafenib, 6–1000 μg/L for cobimetinib, 300–2000 μg/L for niraparib, 10–4000 μg/L for dabrafenib and 100–5000 μg/L for cabozantinib, respectively.

3.2.2 | Selectivity and carryover

Multiple reaction monitoring traces of all six blank EDTA plasma samples from individual donors showed the absence of interference as responses were <20% of the LLOQ and 5% of the IS. Chromatograms of all analytes at the LLOQ level and their respective blank human EDTA sample are shown in Figure 3.

Carryover in the blank sample after injection of the HLOQ sample was <20% of the LLOQ for each drug and <5% of the IS.

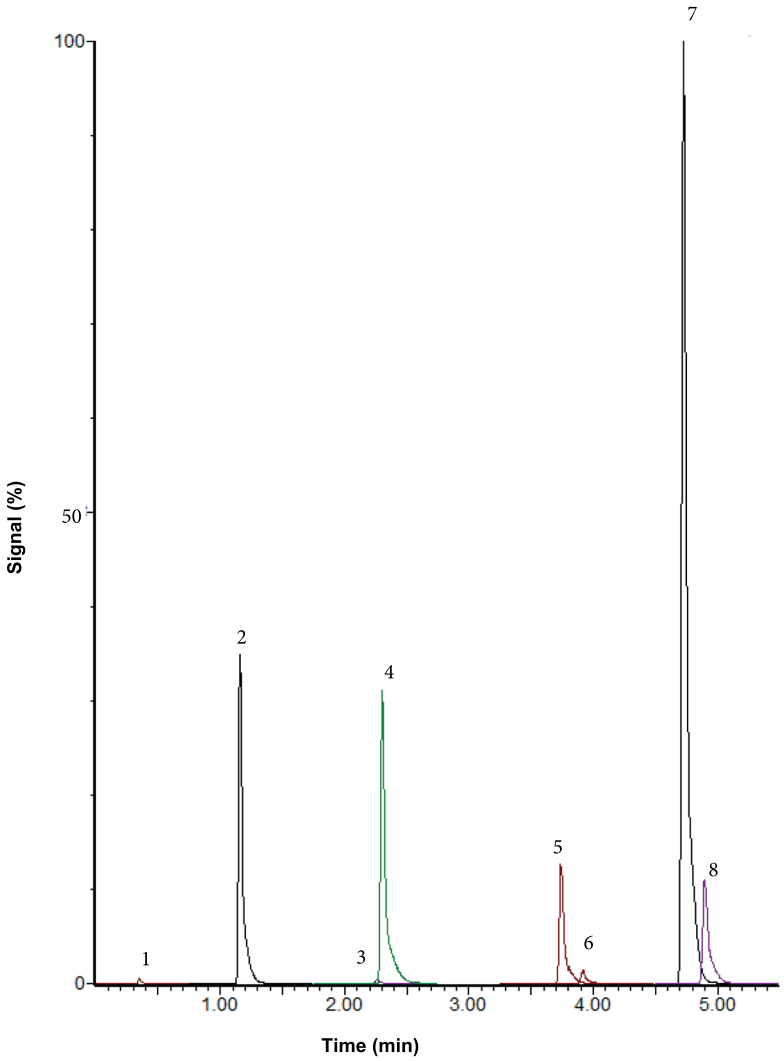

FIGURE 2 Representative reconstructed ion chromatogram overlay of a mixture of the medium quality control samples. 1, niraparib; 2, olaparib; 3, cobimetinib; 4, cabozantinib; 5,dabrafenib; 6, regorafenib M2; 7, vemurafenib; 8, regorafenib

3.2.3 | Accuracy and precision

As presented in Table 3, the accuracy and the within- and between-day precision over the calibration range (LLOQ, QCXL, QCL, QCM, QCH and HLOQ) met the requirements of a RSD <20% for the LLOQ and a RSD <15% for all other concentrations. Within-day accuracy values for all analytes ranged from 86.8 to 115.0% with a precision <10.4%. Between-day accuracy values ranged between 89.7 and 111.9% with a within-day precision <7.4%.

3.2.4 | Recovery

The total extraction recovery ratios, with protein precipitation used for sample preparation, were >70% and constant over the range of concentrations for all analytes.

3.2.5 | Dilution integrity

Two- and 4-fold diluted samples of 1.5*HLOQ were quantified for all analytes. The accuracy for both dilutions ranged from 99.0 to 112.6% for all analytes, except for regorafenib M2 and the 4-fold dilution of dabrafenib. An accuracy of 116.4 and 119.5% was observed for the 2- and 4-fold dilution of regorafenib M2, respectively. For dabrafenib the accuracy for the 4-fold dilution was 125.2%. The precision was <3.0% for all analytes. Consequently, dilution integrity was validated for both dilutions of RGF, OPR, VMF, CBT, NRP, CBZ, only the 2-fold dilution of DBF and not for the dilutions of RGF M2.

3.2.6 | Matrix effect

The CV of the IS-normalized matrix effect calculated from the six plasma batches at both concentrations (QCL, QCXL for cabozantinib, and QCH) was <8.2% for all analytes.

3.2.7 | Stability

Short-term stability of spiked plasma samples was found to be stable after storage at 4°C and room temperature for at least 14 days. Stability analysis for sample handling showed that samples were stable for

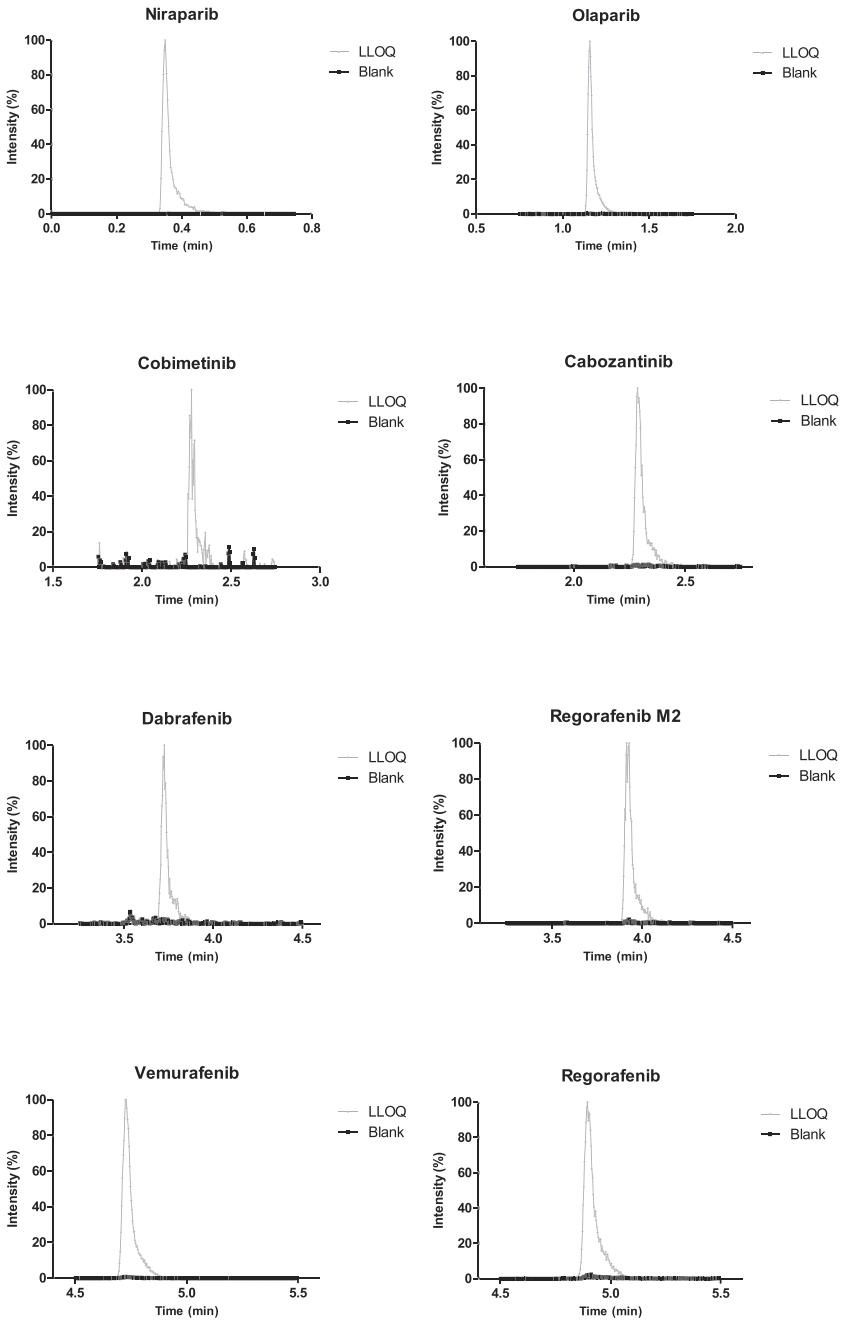

FIGURE 3 Reconstructed ion chromatogram of the lower limit of quantification (LLOQ) and their blank for all eight analytes

at least three freeze–thaw cycles. Processed samples were stable for at least 9 days in the autosampler (4°C).

Long-term stability of the spiked plasma samples stored at −40°C was proven for at least 20 weeks. Stock solutions stored at −40°C remained stable for at least 4.8 months. Samples of patients treated with cabozantinib (n = 2), dabrafenib (n = 6), olaparib (n = 2),niraparib (n = 2) or vemurafenib (n = 1) were stable at −40°C for at least 111, 132, 120, 183 and 132 days, respectively. Stability data are presented in Tables 4 and 5.

3.3 | Clinical application

This validated assay is routinely used in our clinic for pharmacokinetic monitoring in patients with cancer. For the anticancer drugs with well-defined target trough levels, therapeutic drug monitoring is implemented as routine service. For anticancer drugs without an established exposure–response relationship, pharmacokinetic evaluation may be performed occasionally for efficacy, toxicity and/or compliance concerns. Our clinic was consulted to determine whether there was sufficient exposure in a patient with progressive disease during treatment with olaparib 400 mg capsules twice daily. The plasma concentration–time curve for olaparib in this patient is included in Figure 4. Pharmacokinetic parameters were comparable with the pivotal registration data (Mateo et al., 2016), confirming adequate exposure. Reconstructed ion chromatograms of patients samples and the internal standard for cabozantinib, olaparib, niraparib and vemurafenib have been included in Supplementary Figure 1.

TABLE 3 Assay performance data of all eight compounds in human plasma

			Within-day (n = 5)		Between-day (n = 15)	
Drug or metabolite		Concentration (μg/L)	Accuracy (%)	Precision (CV%)	Accuracy (%)	Precision (CV%)
RGF	LLOQ	499.9	104.6	2.2	99.6	4.4
	L	1501.5	105.6	1.6	103.2	2.3
	M	4505.5	103.0	1.2	100.9	1.9
	H	7505.5	97.8	0.9	99.3	1.6
	HLOQ	9998.0	101.9	1.0	100.8	0.9
RGF M2	LLOQ	499.8	91.3	5.6	98.5	7.4
	L	1500.8	108.9	4.5	104.9	3.3
	M	4502.3	104.8	4.6	104.4	0.0
	H	7503.8	97.0	2.4	100.3	2.8
	HLOQ	9996.0	109.1	2.6	103.2	5.2
OPR	LLOQ	199.8	103.0	2.2	100.2	2.5
	L	599.7	102.3	1.7	101.3	0.6
	M	9595.0	102.0	1.0	100.9	1.0
	H	14,992.2	98.8	1.3	99.3	0.1
	HLOQ	19,975.2	101.3	1.4	100.4	0.6
NPR	LLOQ	300.1	115.0	4.0	111.9	2.4
	L	449.8	107.5	2.0	107.0	0.0
	M	839.7	103.5	2.8	102.6	0.0
	H	1499.4	98.5	2.5	99.4	0.7
	HLOQ	2000.6	98.5	1.4	98.6	0.0
CBZ	LLOQ	99.9	92.3	6.1	97.3	4.0
	XL	300.0	106.6	3.2	104.9	1.1
	L	1499.4	103.4	1.3	101.9	1.6
	M	1999.2	97.8	1.7	99.1	1.5
	H	3758.5	97.4	1.8	98.6	1.7
	HLOQ	4997.0	102.3	1.2	100.1	2.1
VMF	LLOQ	4998.70	96.8	1.9	98.1	1.4
	L	14,994.9	103.1	1.0	101.0	1.9
	M	43,985.0	101.9	1.3	100.1	1.5
	H	74,974.5	97.9	0.9	98.3	0.3
	HLOQ	99,974.0	103.3	1.6	101.3	1.7
CBT	LLOQ	6.0	92.8	9.4	98.7	4.4
	L	18.0	91.9	10.4	97.1	3.6
	M	440.0	97.5	2.8	99.5	1.6
	H	740.1	98.0	1.8	98.6	0.0
	HLOQ	1000.1	98.9	2.0	99.8	0.3
DBF	LLOQ	10.0	86.8	4.4	89.7	5.3
	L	40.0	100.7	3.5	100.3	0.0
	M	1499.9	109.0	2.2	107.6	1.0
	H	2999.7	90.2	1.4	92.7	2.3
	HLOQ	3999.2	97.0	1.5	99.3	2.3

Abbreviations: LLOQ, lower limit of quantification; L, low; M, medium; H, high; XL, extra low.
In cases where the between-day imprecision is 0.0%, no additional variation upon the within-day imprecision is observed as a result of performing the assay on different days.

TABLE 4 Stability of spiked samples at various conditions

Matrix	Condition	Component	Time interval (days)	Mean concentration compared with nominal concentration (%)
Spiked EDTA plasma[a]	4°C	RGF	14	104.3
		RGF M2	14	96.5
		OPR	14	102.0
		NRP	14	103.6
		CBZ	14	103.6
		VMF	14	103.5
		CBT	14	100.8
		DBF	14	100.9
Spiked EDTA plasma[a]	Room temperature	RGF	14	103.1
		RGF M2	14	97.5
		OPR	14	102.1
		NRP	14	93.3
		CBZ	14	103.3
		VMF	14	101.2
		CBT	14	95.9
		DBF	14	101.8
Spiked EDTA plasma[a]	−40°C	RGF	141	101.3
		RGF M2	141	101.0
		OPR	141	97.2
		NRP	141	94.8
		CBZ	141	98.4
		VMF	141	98.5
		CBT	141	91.2
		DBF	141	96.8
Spiked EDTA plasma[a]	Three freeze–thaw cycles	RGF	–	102.9
		RGF M2	–	95.6
		OPR	–	101.6
		NRP	–	104.7
		CBZ	–	100.9
		VMF	–	102.2
		CBT	–	97.1
		DBF	–	99.7

[a]Mean recovery of the concentration range QC low to QC high (n = 6) for each analyte.

4 | DISCUSSION

In this paper we described the development, validation and application of a UPLC–MS/MS method for the quantification of vemurafenib, cobimetinib, dabrafenib, cabozantinib, regorafenib plus metabolite regorafenib M2, niraparib and olaparib. To our knowledge, this is the first method which measures olaparib and niraparib in combination with the above-mentioned analytes in a single run.

Numerous methods have been developed for the quantification of combinations of tyrosine kinase inhibitors. One of the main difficulties of the analysis of multiple tyrosine kinase inhibitors is the large difference in clinically relevant concentrations for some of them. Vemurafenib has a target trough concentration of 42,000 µg/L which is several times higher than the levels of other tyrosine kinase inhibitors included in our method (Goldwirt et al., 2016). Quantification methods of vemurafenib combined with other tyrosine kinase inhibitors that have been previously published, use solid-phase extraction (Rousset et al., 2017), protein precipitation with methanol followed by a step of evaporation (Cardoso et al., 2018) and protein precipitation with acetonitrile and zinc-sulfate for sample preparation (Huynh et al., 2017). An advantage of our analytical method is the simple sample preparation by protein precipitation with acetonitrile. Although the sample preparation is simple, limited matrix effects were observed by this approach. The sample volume of 50 µl is equal to or less than that

TABLE 5 Stability of processed samples in the autosampler and stock solutions

Matrix	Condition	Component	Nominal concentration (µg/L)	*n*	Time interval	Mean concentration compared with nominal concentration (%)
Processed plasma	Autosampler 4°C[a]	RGF	1500	5	9 days	102.7
			7500	5		98.8
		RGF M2	1500	5	9 days	98.4
			7500	5		97.4
		OPR	600	5	9 days	101.3
			15,000	5		99.5
		NRP	450	5	9 days	106.4
			1500	5		100.0
		CBZ	500	5	9 days	99.6
			3760	5		97.6
		VMF	15,000	5	9 days	100.6
			75,000	5		97.5
		CBT	18	5	9 days	98.6
			740	5		96.3
		DBF	40	5	9 days	100.6
			3000	5		94.7
DMSO (stock solution)	−40°C	RGF	1000[b]	3	4.8 months	102.7
		RGF M2	1000	3	4.8 months	99.5
		OPR	10,000[b]	3	4.8 months	101.0
		NRP	1000[b]	3	4.8 months	103.1
		CBZ	1000[b]	3	4.8 months	100.3
		VMF	10,000[b]	3	4.8 months	97.5
		CBT	1000[b]	3	4.8 months	100.8
		DBF	1000[b]	3	4.8 months	91.7

[a]Autosampler stability was tested at 4°C in order to facilitate batch preparation in advance.
[b]Concentrations in mg/L.

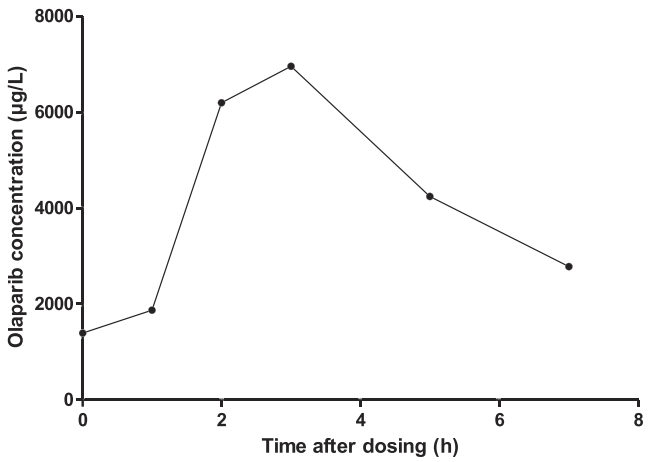

FIGURE 4 Steady-state plasma concentration–time curve of olaparib in a patient treated with 400 mg capsules twice daily

in previously published methods (Abdelhameed et al., 2017; Cardoso et al., 2018; Huynh et al., 2017; Luethi et al., 2014; Merienne et al., 2018; Nijenhuis et al., 2013; Nijenhuis et al., 2014; Pressiat et al., 2018; Rousset et al., 2017; van Andel et al., 2017; van Erp et al., 2013).

Another major difficulty of the analysis of multiple oral oncolytics in a single run is the wide variety in chemical characteristics of these drugs as shown in Figure 1. For this reason, almost all of the reported multiple tyrosine kinase inhibitors use a gradient elution system (Cardoso et al., 2018; Huynh et al., 2017; Merienne et al., 2018; Pressiat et al., 2018; van Erp et al., 2013). Niraparib has a relatively hydrophilic structure and has not yet been included in a multianalyte assay to our knowledge. In our method the analytes are separated adequately to quantify all eight analytes with the use of a gradient system combined with a Cortecs UPLC C18 column. This column tolerates a flow of 0.8 ml/min, which facilitates the relatively short run time of 7 min within the range of previously described runs with multiple tyrosine kinase inhibitors of ~5–10 min (Abdelhameed et al., 2017; Cardoso et al., 2018; Huynh et al., 2017; Merienne et al., 2018; Pressiat et al., 2018; van Erp et al., 2013).

An important limitation of our method is the need for a set of nine working solutions to reach the range for the calibration standards, which is a labour-intensive approach. However, our method

enables simultaneous quantification of eight chemically diverse oral targeted anticancer drugs with a wide range of clinical concentrations and is therefore suitable for application in the clinical setting.

In conclusion, we have developed and validated a robust and UPLC–MS/MS method for the simultaneous quantification of seven new oral anticancer drugs. The assay is used for both guidance of individual patients and for clinical pharmacological trials in our clinic.

CONFLICT OF INTEREST

SK, EvMT, FJ, DB and NvE declare that they have no conflicts of interest that are directly relevant to the content of this manuscript.

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SUPPORTING INFORMATION

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